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Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters

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Summary

The lack of an effective evaluation method for salt tolerance in the screening process is one of the reasons for limited success in conventional salt tolerance breeding. This study was designed to identify useful agronomic parameters for evaluation of salt tolerance and to evaluate genotypes by multiple agronomic parameters for salt tolerance at different growth stages. Twelve genotypes were grown in a greenhouse in sand and irrigated with nutrient solutions of control and treatments amended with NaCl and $CaCl_2$ (5:1 molar concentration) at 4.4 and 8.2 dS m^{-1} electrical conductivity. Wide genotypic differences in relative salt tolerance based on seedling growth were identified. The duration of reproductive growth between panicle initiation and anthesis was either reduced or increased by salinity, but the response was not strictly correlated with relative salt tolerance in seed yield among genotypes. Wide genotypic differences in relative salt tolerance based on spikelet and tiller numbers were identified. Few genotypic differences were identified for fertility and kernel weight. Spikelet and tiller numbers contributed most of the variation to seed yield among parameters investigated. When genotypes were ranked for salt tolerance based on the means of multiple parameters, dramatic changes of salt tolerance at early and seed maturity stages were observed in two genotypes, GZ5291-7-1-2 and GZ178. IR63731-1-1-4-3-2 was identified with a favourable combination of salt tolerance at early seedling and seed maturity stages. Cluster group ranking of genotypes based on multiple agronomic characters can be applied in salt tolerance breeding to evaluate salt tolerance and may have great advantage over conventional methods.

Abbreviations: DAP – day after planting; PI – panicle initiation

Introduction

Salinity is one of the major obstacles to increasing crop production worldwide. The existing salinity problems in crop production will become worse due to rapidly growing human population in many countries and the increasing concerns over the limited water resources which are forcing growers to use poor quality water for irrigation. Rice (*Oryza sativa* L.) is rated as one of the major food crops in the world, but is also considered extremely salt-sensitive (Maas & Hoffman, 1977). In California, where direct water-seeded systems are dominant, salinity problems in rice production are mainly caused by irrigation practices during the early growth stages (Scardaci et al., 1996). The strategies for mitigating salinity problems in crop production include both development of management options (Shannon, 1997) and genetic improvement of salinity tolerance in current cultivars (Epstein et al., 1980). Although the use of some management options can ameliorate yield reduction under salinity stress, implementation is often limited because of cost and availability of good quality water resources. Therefore, the need for genetic improvement of salt tolerance is great and is expected to increase dramatically in the future.

In conventional breeding, i.e., the approaches based on genetic variation existing for the character

in the gene pool, screening for genetic diversity in agronomic characters within extant genotypes is the first step toward the genetic improvement of crops. Screening for salt tolerance has been accomplished in a number of crops for some genotypes (Dewey, 1962; Shannon, 1978; Grieve et al., 1999; Royo & Aragues, 1999). In rice, a tremendous number of genotypes have been screened for salt tolerance. Some seedling evaluation methods have been used for mass screening of seedlings at the International Rice Research Institute (Akbar, 1985; IRRI, 1996; Gregorio et al., 1997). These methods were designed to screen for salt tolerance based on plant vigour (i.e., plant growth at early growth stages) or visual damage on vegetative tissues. Success in the application of these methods in salt tolerance breeding has been limited. For example, the efforts in mass screening for plant vigour have only identified the cultivars or breeding lines with non-dwarf plant type (Yeo et al., 1990). Furthermore, the use of visual damage as evaluation for salt tolerance is not always applicable because the symptoms such as chlorosis and leaf rolling are not always easily observed in rice at low or moderate salinity. Alternatively, screening for genetic diversity in physiological characters can be an effective approach in salt tolerance breeding (Yeo & Flowers, 1986; Yeo et al., 1990). This approach has proved successful in an international cooperative project which has developed a salt tolerant cultivar, CSR10, in India (IRRI, 1997). However, the utilization of physiological characters in salt tolerance breeding in no way reduces the significance of agronomic characters in such a program. Instead, the methods for evaluating agronomic characters in salt tolerance screening should be improved since these characters, especially seed yield, are always the primary targets in plant breeding.

The lack of an effective evaluation method for salt tolerance in the screening of genotypes is one of the reasons for the limited success in conventional salt tolerance breeding. Two yield parameters, tiller number per plant and spikelet number per panicle, have proved most sensitive to salinity and are highly significantly correlated to final seed yield in cultivar M-202 under salt stress (Zeng & Shannon, 2000a, b). However, it is still necessary to identify salinity-sensitive agronomic parameters among diverse genotypes. Furthermore, the method of ranking genotypes for salt tolerance, especially when multiple parameters are involved, has to be improved. Genotypes often have been evaluated on the basis of multiple morphological characters, especially yield parameters because most of them are significantly correlated with each other. Compensatory relationships have been identified among tiller number, spikelet number, and fertility in rice (Counce & Wells 1990; Gravois & McNew, 1993; Kato & Takeda, 1996). In conventional methods, genotypes are usually scored and ranked on single characters. An appropriate statistical method will be helpful to analyze multiple agronomic parameters simultaneously in the evaluation of genotypes and facilitate the scores and rankings for salt tolerance among genotypes. The application of cluster analysis in multivariate observations has been suggested for comparisons of cultivar means (Jolliffe et al., 1989). However, only one application has been reported using multivariate analysis in the screening of *in vitro* cultures for salt tolerance in potato (Khrais et al., 1998).

The effects of salinity on plants are complex and easily modulated by environmental conditions such as temperature and humidity (Shannon, 1997). It would be difficult to determine the critical parameters under field conditions since any environmental change could result in dramatic change in the plant's response to salinity. This study was designed to evaluate salt tolerance among genotypes under greenhouse conditions. The identified parameters and evaluation method for salt tolerance can then be applied to breeding practices under field conditions. The objectives of this study were to (a) identify agronomic parameters which are sensitive to salinity, diverse among genotypes for salt tolerance, and contribute to salt tolerance in terms of seed yield; (b) evaluate genotypes for relative salt tolerance of multiple agronomic parameters at different growth stages using multivariate analysis.

Materials and methods

Plants

Seeds of 12 rice genotypes were received from Field Crops Research Institute, Giza, Egypt; International Rice Research Institute (IRRI), the Philippines; and California Cooperative Rice Research Foundation Inc., Biggs, CA. The genotypes, GZ177 and GZ178 from Egypt, Agami from IRRI, and M103, M201 and M202 from California, are commercial cultivars. The remainders are breeding lines: AC26, GZ1368-5-4, GZ5291-7-1-2, IR71657-5B-B–12PB, IR50184-3B-18-2B-1, and IR63731-1-1-4-3-2. These genotypes were a subset of the germplasm collections from the three regions with different reputations for

salt tolerance. For example, GZ1368-5-4 is an elite salt tolerant breeding line (A.T. Badawi, personal communications, Field Crops Research Institute, Giza, Egypt) while common cultivars in California have limited salt tolerance (Shannon et al., 1998).

Plant culture and experimental design

Two trials were conducted in the greenhouse at Riverside, CA [33°58′24″ N latitude, 117°19'12″ W longitude]. The first trial was between March and July 1999. The second one was between July and November 1999. The plants were cultured in nutrient solution (Yoshida et al., 1971) in sand tanks (122 × 61 × 46 cm deep) filled with sand (#12, Cisco Inc., CA)[1] with an average bulk density of 1.4 g cm^{-3}. Nutrient solution pH was maintained between 5.0 to 6.5 by adding sulfuric acid twice a week. Irrigation solutions were prepared in 1600 L reservoirs and pumped to provide irrigation to the sand tanks. Overflow irrigation was returned to the reservoirs through drainage by gravity. Each reservoir provided irrigation to six sand tanks (replicates) three times daily for 30 min. per irrigation cycle. Seeds were planted in four rows per genotype and four genotypes per tank. The rows were spaced 7 cm apart with 20 seeds per row. Sowing depth was less than 1 cm. Water depth was controlled at 1 to 2 cm during the first week and at 6 to 8 cm thereafter. Air temperature ranged from 25 to 33 °C during the day and 18 to 23 °C during the night. Humidity ranged from 40 to 85%. Light averaged 671 μmol·m^{-2}·s^{-1} with a minimum of 100 and a maximum 1400 μmol·m^{-2}·s^{-1} during the day. The experiment was designed as a randomized block in a split-plot with six replicates. Salt level was a main plot factor and genotype was a sub-plot factor.

Salinity treatments

NaCl and $CaCl_2$ (5:1 molar concentration) were added to the nutrient solutions on the first day after planting (DAP). Salinity was maintained continuously until final harvest. Electrical conductivities (EC_w) of nutrient solutions were measured with an electrical conductivity metre on alternate days. Over the duration of stress, the first salt level (designated as a moderate salt level) averaged EC_w of 4.5 dS m^{-1} (4.1–4.8) during Trial 1 and 4.2 dS m^{-1} (4.0–4.5) during Trial 2. The second salt level (designated as a high salt level) averaged EC_w of 8.3 dS m^{-1} (7.5–8.9) during Trial 1 and 8.1 dS m^{-1} (7.1–8.5) during Trial 2. For convenience, EC_w of 4.4 and 8.2 dS m^{-1} averaged over the two trials were used to represent moderate and high salt levels, respectively. The control, i.e., nutrient solution without added salts, had an EC_w of 0.9 dS m^{-1} during the two trials.

Growth stages

Plant growth stages were measured and recorded using thermal time (°C·d, i.e., thermal degree day) (Logan & Boyland, 1983), assuming a base temperature of 10 °C. Two plants were randomly chosen from each treatment every day when plants were approaching panicle initiation (PI). The main culms were dissected under a dissecting microscope to observe the development of young panicles. The first day that a young panicle reached 0.5 mm in actual length in any plant dissected for each treatment, was defined as the age of PI. Anthesis was defined as the stage when the first spikelet began to flower in a panicle. The durations between PI and anthesis were compared among genotypes using Duncan's multiple range test (Ott, 1993).

Measurements at the seedling stage

Seedling survival rate was measured at 327 °C·d (i.e., 24 DAP). The seedling survival rate was calculated as the percentage of live seedlings from germinated plants. Ten seedlings from each replicate were randomly sampled from surviving plants at 340 °C·d (i.e., 25 DAP). After roots were removed, shoots of seedlings were dried in a forced-air oven (70 °C) and then measured for dry weights. Data were averaged over the ten subsamples. After the seedling harvest, dead plants were removed and the remaining plants in each sand tank were thinned to 7 cm between rows and 6 to 7 cm between plants within the rows. Visual damage on plants, i.e., chlorosis and leaf rolling, was observed and recorded for each genotype during the trials.

Measurements at the seed maturity stage

When seeds on primary tillers matured (i.e., the kernels were too hard to be dented by the thumbnail), eight plants from each replicate were harvested by pulling up the roots. Plants were bagged individually after roots were removed. After oven-drying at

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

70 °C, seeds from each panicle of four plants among the eight plants harvested were counted and weighed. Data were averaged across all panicles to determine spikelet number per panicle, fertility (i.e., percentage of filled spikelets among total number of spikelets per panicle), and kernel weight (i.e., the weight per single seed). The data were averaged across the four subsamples. All matured panicles were hand-threshed and weighed separately from the rest of the plants harvested. The data were combined with the previous four plants to determine seed weight per panicle and seed weight per plant. Tiller numbers of each of the eight harvested plants were determined for all primary tillers with emerged heads.

Ranking of genotypes for salt tolerance

In conventional methods to allow comparisons, genotypes are usually scored based on the measurements of a single morphological character. Score boundaries or intervals have to be set based on the ranges of the observations. This process is often inaccurate, artificial, and cumbersome especially when a large number of genotypes and multiple characters for each genotype must be screened. Using cluster analysis, genotypes can be scored on multiple parameters simultaneously. Furthermore, there is no need to set score boundaries since genotypes are scored based on cluster group rankings. In doing so, genotypes are grouped for their responses to salinity, e.g., spikelet and tiller numbers under salt stress, such that the genotypes within a group tend to be similar to each other and dissimilar among groups for their yield potentials under stress. Cluster group ranking numbers can be assigned to cluster groups based on cluster means. Genotypes are then scored based on cluster group ranking numbers.

All the data were converted to relative values, i.e., salt tolerance indexes before cluster analysis. Salt tolerance index was defined as the observations under salinity divided by the means of the controls. Cluster analysis followed the methods described by Jolliffe et al. (1989) and Khrais et al. (1998). Cluster group rankings were obtained based on Ward's minimum-variance cluster analysis on the means of the salt tolerance indexes for two parameters at the seedling stage, i.e., seedling growth and survival, and two parameters at the seed maturity stage, i.e., spikelet number per panicle and tiller number per plant. The distance between two clusters was calculated as the ANOVA sum of squares between the two clusters in all the parameters analyzed. The clusters were merged in each generation to minimize the within-cluster sum of squares. The procedures are described in the SAS User's Guide (SAS Institute, 1994). The cluster groups were identified in dendrograms. The number of cluster groups was determined by calculating the pseudo t^2 which reached a local maximum. The cluster group rankings were obtained from the averages of means over multiple parameters in each cluster group, i.e., cluster mean, in order from highest to lowest averages. A sum was obtained by adding the numbers of cluster group ranking at each salt level in each genotype. The genotypes were finally ranked based on the sums in order that those with the smallest sums were ranked as the most tolerant and those with the largest sums were ranked as the least tolerant in terms of relative salt tolerance.

Results

Generally, seedling shoot dry weight at 340 °C·d and survival rate decreased with increasing salinity. However, relative salt tolerance in terms of these two parameters varied among genotypes (Table 1). The salt tolerance indexes of seedling shoot dry weight ranged from 0.53 to 0.99 at 4.4 dS m^{-1}, and 0.49 to 0.89 at 8.2 dS m^{-1}. The salt tolerance indexes of seedling survival ranged from 0.71 to 0.96 at 4.4 dS m^{-1}, and 0.67 to 0.91 at 8.2 dS m^{-1}. Genotypes were divided into four cluster groups at 4.4 dS m^{-1} and five cluster groups at 8.2 dS m^{-1} by simultaneous analysis on salt tolerance indexes in seedling shoot dry weight and survival rate using Ward's minimum-variance cluster analysis (Table 2). The genotypes were finally ranked with IR63731-1-1-4-3-2 as the most tolerant and GZ178 as the least tolerant in terms of seedling growth. During the trials, visual damage on seedling leaves was not obvious at 4.4 dS m^{-1} although chlorosis was occasionally observed on some plants. At 8.2 dS m^{-1}, visual damage was serious; chlorosis and leaf rolling were observed on all plants. Although the damage on IR63731-1-1-4-3-2 and IR50184-3B-18-2B-1 appeared less than the other genotypes, the scores among genotypes based on visual estimation were difficult to quantify and did not develop into rankings among genotypes (data not shown).

Reproductive growth was delayed by salinity in all genotypes (Table 3). PI was delayed in all genotypes except in Agami at 4.4 dS m^{-1}. Anthesis was delayed in all genotypes at the highest salt level. At moderate salinity, anthesis was not delayed in M-103, M-201,

Table 1. Salt tolerance indexes[a] of agronomic parameters in rice under different levels of salinity. Data were averages from Trial 1 and Trial 2 conducted in 1999

Genotype	Salt level (dS m^{-1})	Seedling shoot wt.	Seedling survival	Seed yield	Seed wt per panicle	Spikelets per panicle	Tillers per plant	Fertility	Kernel wt.
GZ177	4.4	0.68	0.96	0.64	0.73	0.74	0.70	1.00	0.97
	8.2	0.57	0.88	0.18	0.44	0.68	0.50	0.71	0.87
GZ178	4.4	0.53	0.72	0.70	0.79	0.74	0.86	0.94	1.14
	8.2	0.52	0.67	0.26	0.44	0.67	0.62	0.66	1.10
IR71657-5R-B-12PB	4.4	0.72	0.71	0.41	0.54	0.58	0.65	0.89	0.97
	8.2	0.73	0.70	0.21	0.27	0.44	0.68	0.58	1.07
M-103	4.4	0.73	0.88	0.46	0.69	0.57	0.69	0.93	1.24
	8.2	0.61	0.75	0.17	0.39	0.37	0.50	0.88	1.22
AC26	4.4	0.83	0.92	0.56	0.73	0.69	0.72	0.98	1.09
	8.2	0.68	0.86	0.29	0.47	0.48	0.61	0.84	1.16
GZ1368-5-4	4.4	0.79	0.93	0.79	0.74	0.70	0.92	0.94	1.06
	8.2	0.68	0.79	0.44	0.47	0.58	0.97	0.71	1.14
Agami	4.4	0.78	0.89	0.81	0.78	0.76	1.05	1.01	1.05
	8.2	0.70	0.79	0.47	0.45	0.65	1.01	0.72	1.04
M-201	4.4	0.70	0.90	0.48	0.66	0.67	0.67	0.96	1.00
	8.2	0.49	0.73	0.16	0.29	0.47	0.53	0.66	1.09
GZ5291-7-1-2	4.4	0.83	0.95	0.55	0.69	0.70	0.68	0.97	0.96
	8.2	0.72	0.89	0.20	0.36	0.50	0.48	0.72	0.94
IR50184-3B-18-2B-1	4.4	0.82	0.96	0.70	0.80	0.75	0.89	0.95	0.97
	8.2	0.75	0.91	0.32	0.41	0.62	0.75	0.70	1.02
IR63731-1-1-4-3-2	4.4	0.99	0.95	0.81	0.79	0.84	1.03	0.93	1.08
	8.2	0.89	0.88	0.54	0.53	0.76	1.05	0.80	0.89
M-202	4.4	0.75	0.87	0.54	0.81	0.84	0.66	0.96	1.08
	8.2	0.59	0.78	0.12	0.21	0.40	0.57	0.47	1.05

[a] Salt tolerance index was defined as the observations under salinity divided by the means of the controls.

GZ1368-5-4, Agami, and IR71657-5R-B-12PB. The duration between PI and anthesis was significantly reduced in M-103, M-201, GZ178, and IR71657-5R-B-12PB, not significantly reduced in M-202, AC26 and GZ177, and significantly increased in GZ5291-7-1-2, GZ1368-5-4, Agami, and IR63731-1-1-4-3-2 under salinity when compared to the controls (Table 3).

Generally, the salt tolerance indexes in terms of seed yield (i.e., seed weight per plant), seed weight per panicle, spikelet number per panicle, and tiller number per plant were reduced with increasing salinity (Table 1). Tiller number per plant, however, was not reduced by salinity in GZ1368-5-4, Agami, and IR63731-1-4-3-2. The salt tolerance indexes of spikelet number per panicle ranged from 0.57 to 0.84 at 4.4 dS m^{-1}, and from 0.37 to 0.76 at 8.2 dS m^{-1} among genotypes. Generally, fertility was not reduced by salinity at 4.4 dS m^{-1}, but reduced at 8.2 dS $^{-1}$ in all genotypes (Table 1). In contrast, the salinity effect was not significant for kernel weight in most genotypes (Table 1). In the analysis of the relationships between seed yield and the other parameters, spikelet number per panicle and tiller number per plant contributed the most variation to seed yield when data from all genotypes were combined (Table 4).

Based on simultaneous analysis on the means of salt tolerance indexes in spikelet number per panicle and tiller number per plant using Ward's minimum-variance cluster analysis, the genotypes were divided into four cluster groups at moderate salinity and three cluster groups at high salinity (Table 5). Genotypes were finally ranked with IR63731-1-1-4-3-2, Agami and GZ1368-5-4 as the most tolerant, and M-103 and IR71657-5R-B-12PB as the least tolerant among all genotypes (Table 5).

Table 2. Rankings of genotypes for their relative salt tolerance in terms of seedling growth (seedling shoot dry weight and survival) in a cluster analysis (Ward's minimum variance analysis)

Genotype	Salt level (dS m^{-1})	Cluster group[a] ranking	Sum[b]	Genotype[c] ranking
IR63731-1-1-4-3-2	4.4	1	2	1
	8.2	1		
GZ5291-7-1-2	4.4	2	4	2
	8.2	2		
IR50184-3B-18-2B-1	4.4	2	4	2
	8.2	2		
AC26	4.4	2	4	2
	8.2	2		
Agami	4.4	3	5	3
	8.2	2		
GZ1368-5-4	4.4	2	6	4
	8.2	4		
GZ177	4.4	3	7	5
	8.2	4		
R71657-5R-B-12PB	4.4	4	7	5
	8.2	3		
M-202	4.4	3	7	5
	8.2	4		
M-103	4.4	3	7	5
	8.2	4		
M-201	4.4	3	8	6
	8.2	5		
GZ178	4.4	4	9	7
	8.2	5		

[a] Cluster groups were obtained from Ward's minimum-variance cluster analysis on the means of the salt tolerance indexes in seedling shoot dry weight and seedling survival (see Material and Methods). Genotypes were divided into four cluster groups at 4.4 dS m^{-1} and five cluster groups at 8.2 dS m^{-1}. The cluster group rankings were obtained from cluster means (data not shown) in the order from the highest to the lowest cluster means.
[b] Sums were obtained from the cluster group rankings by adding the ranking numbers at the two salt levels in each genotype.
[c] Genotypes were finally ranked based on the sums with the smallest sum being the most relatively tolerant.

Discussion

Rice plants are very sensitive to salinity during the seedling stage (Pearson & Bernstein, 1959; Flowers & Yeo, 1981). Low salinity threshold values of rice seedling growth and survival rate have been reported for cultivar M-202 (Zeng & Shannon, 2000a). The loss of plant stand caused reduction in yield sink capacity by reducing plant density. Therefore, screening of genotypes for salt tolerance based on these two agronomic parameters, seedling growth and survival rate, is important for the improvement of rice production under salinity, especially in those regions where direct-water seeding systems are dominant. However, salt tolerance at early growth stages does not always correlate with that at seed maturity stages. Changes in salt tolerance at different growth stages were observed in rice (Pearson & Bernstein, 1959; Lutts et al., 1995) and other species such as corn (*Zea mays*) (Maas et al., 1983) and cowpea (*Vigna unguiculata* L.) (Maas & Poss, 1989). Therefore, in this study, genotypes were screened for salt tolerance at different growth stages.

Previous screening of the common rice cultivars in California has shown that genotypic differences for salt tolerance on seedling growth were small (Shannon et al., 1998). In the present screening experiment, wide

Table 3. Reproductive development of rice affected by salinity in different genotypes (Trial 1, 1999)

Genotype	Salt level (dS m^{-1})	PI[a] °C·d (DAP)	Delay[b] °C·d	Anthesis °C·d (DAP)	Delay °C·d	Duration[c] °C·d
M-103	0.9	608 (44)		1067 (75)		459a
	4.4	635 (46)	27	1067 (75)	0	432ab
	8.2	732 (53)	124	1142 (80)	75	410b
M-201	0.9	635 (46)		1173 (82)		538a
	4.4	689 (50)	54	1173 (82)	0	484b
	8.2	759 (55)	124	1215 (85)	40	456b
M-202	0.9	617 (45)		1142 (80)		525a
	4.4	658 (48)	41	1177 (82)	35	519a
	8.2	750 (54)	133	1286 (90)	144	536a
AC26	0.9	608 (44)		1040 (73)		432a
	4.4	635 (46)	27	1067 (75)	27	427a
	8.2	760 (55)	152	1172 (82)	132	412a
GZ177	0.9	689 (50)		1067 (75)		378a
	4.4	717 (52)	28	1127 (79)	60	410a
	8.2	843 (61)	154	1200 (84)	133	357a
GZ5291-7-1-2	0.9	635 (46)		1067 (75)		432b
	4.4	689 (50)	54	1172 (82)	106	483a
	8.2	791 (57)	156	1290 (90)	223	499a
GZ1368-5-4	0.9	775 (56)		1492 (104)		717b
	4.4	806 (58)	31	1492 (104)	0	686b
	8.2	848 (61)	73	1642 (113)	161	794a
GZ178	0.9	775 (56)		1492 (104)		717a
	4.4	786 (57)	11	1503 (105)	11	717a
	8.2	906 (65)	131	1518 (106)	31	612b
Agami	0.9	806 (58)		1492 (104)		686b
	4.4	806 (58)	0	1492 (104)	0	686b
	8.2	877 (63)	71	1653 (114)	161	776a
IR63731-1-1-4-3-2	0.9	806 (58)		1701 (117)		895b
	4.4	815 (59)	9	1719 (118)	18	904b
	8.2	892 (64)	86	1835 (124)	134	943a
IR71657-5R-B-12PB	0.9	848 (61)		1820 (124)		972a
	4.4	877 (63)	29	1820 (124)	0	943b
	8.2	966 (69)	118	1903 (129)	83	937c

[a] PI, panicle initiation; °C·d, thermal degree day, assuming based temperature to be 10 °C; DAP, days after planting. The number represented the accumulative thermal degree day (or days after planting) when PI or anthesis was approached.
[b] The delay of accumulative thermal time when PI or anthesis was approached under salinity compared to the controls.
[c] The duration between PI and anthesis recorded by thermal degree day. The numbers followed by the same letter in the column within each genotype are not significantly different at 0.05 significance level based on Duncan's multiple range test.

genotypic differences were identified among the genotypes from different sources. The genotypes IR63731-1-1-4-3-2, GZ5291-7-1-2, IR50184-3B-18-2B-1 and AC26 showed high salt tolerance at seedling growth whereas the others were less tolerant. The decision to incorporate these genotypes in salt tolerance breeding programs depends on the combination of salt tolerance at different growth stages.

Previous research efforts have shown that rice panicle emergence is delayed by salinity (Heenan et al., 1988; Khatun et al., 1995). However, the relationships of the delay in reproductive growth with the salt tolerance based on seed yield have never been shown. In

Table 4. Relationships between seed yield and other agronomic parameters under salinity based on stepwise analysis (Trial 1). Data were combined among genotypes in the regression analysis. Regression equation determined by stepwise analysis: seed yield = –6.99 + 0.95 (spikelets per panicle) + 0.84 (tillers per plant) + 1.02 (fertility) + 0.69 (kernel weight) + 0.21 (reproductive stages)

Relationship to seed yield	Spikelets per panicle	Tillers per plant	Fertility	Kernel wt	Reproductive stage
Correlation (r^2)	0.53	0.43	0.36	0.01	0.05
Partial regression coefficient (r)	0.73	0.41	0.27	0.21	0.08

Table 5. Rankings of genotypes for relative salt tolerance in terms of seed yield and yield components (i.e., spikelets per panicle and tillers per plant) in a cluster analysis (Ward's minimum variance analysis)

Genotype	Salt level (dS m^{-1})	Rankings (yield components)			Rankings (seed yield)		
		Cluster group[a] ranking	Sum[b]	Genotype[c] ranking	Cluster group ranking	Sum	Genotype ranking
IR63731-1-1-4-3-2	4.4	1	2	1	1	2	1
	8.2	1			1		
Agami	4.4	1	2	1	1	2	1
	8.2	1			1		
GZ1368-5-4	4.4	2	3	2	1	2	1
	8.2	1			1		
GZ178	4.4	2	4	3	2	4	2
	8.2	2			2		
IR50184-3B-18-2B-1	4.4	2	4	3	2	4	2
	8.2	2			2		
GZ177	4.4	3	5	4	2	5	3
	8.2	2			3		
M-202	4.4	3	6	5	3	6	4
	8.2	3			3		
AC26	4.4	3	6	5	3	5	3
	8.2	3			2		
GZ5291-7-1-2	4.4	3	6	5	3	6	4
	8.2	3			3		
M-201	4.4	3	6	5	4	7	5
	8.2	3			3		
IR71657-5R-B-12PB	4.4	4	7	6	4	7	5
	8.2	3			3		
M-103	4.4	4	7	6	4	7	5
	8.2	3			3		

[a] Cluster groups were obtained from Ward's minimum-variance cluster analysis on the means of the salt tolerance indexes (see Material and methods). Genotypes were divided into four cluster groups at 4.4 dS m^{-1} and three cluster groups at 8.2 dS m^{-1}. The cluster group rankings were obtained from the cluster means (data not shown) in the order from the highest to the lowest clusters.

[b] Sums were obtained from the cluster group rankings by adding the ranking numbers at the two salt levels in each genotype.

[c] Genotypes were finally ranked based on the sums with the smallest sum being the most relatively tolerant.

our experiment, genotypic differences were identified in the delay of both PI and anthesis. In genotypes such as GZ178, M-201 and IR71657-5R-B-12PB, the delay of PI was more than that of anthesis under salinity. As a result, the duration of reproductive growth between PI and anthesis significantly decreased when compared to the controls in these genotypes. There was no obvious correlation between the duration of reproductive growth and salt tolerance in terms of seed yield because the relative salt tolerance of yield components was dramatically different in these genotypes. In other genotypes such as IR63731-1-1-4-3-2, Agami, and GZ5291-7-1-2, the delay of anthesis was more than that of PI under salinity. As a result, the duration of reproductive growth significantly increased under salinity compared to the controls in these genotypes. Again, the relative salt tolerance of yield components was dramatically different among these genotypes.

These results indicate that the duration of reproductive growth is not unequivocally a major contributor to salt tolerance based on seed yield. If it is true that the correlation between the duration of reproductive growth and salt tolerance is not definite, a loose linkage, but not a tight linkage, exists between the delay of reproductive growth under salinity stress and salt tolerance. Since high salt tolerance is often identified in those breeding lines that have a relatively long growing season, this finding should be a valuable indicator for plant breeders in their efforts to separate the two agronomic characters in segregating populations.

Evaluation for yield potential under salt stress is a critical component of breeding programs since improving seed yield is always the main target in plant breeding. Yield components of rice are sensitive to salinity. Tiller number per plant, spikelet number per panicle, fertility, panicle length, and primary branches per panicle were significantly reduced by salinity in studies by other researchers (Heenan et al., 1988; Cui et al., 1995; Khatun et al., 1995). Spikelet number per panicle was determined to be the most salt sensitive yield component, and reductions in spikelet number per panicle and tiller number per plant were the major causes of yield loss in cultivar M-202 under salinity (Zeng & Shannon, 2000a, b). Generally, in the present experiment, spikelet number per panicle and tiller number per plant were reduced by salinity. However, wide genotypic differences observed for these yield parameters indicate that evaluation for salt tolerance among genotypes can be based on the genetic diversity in spikelet and tiller numbers. In contrast, fertility and kernel weight were less sensitive to salinity. Few genotypic differences were identified among genotypes on these two parameters. The rankings among genotypes for relative salt tolerance based on spikelet and tiller numbers were close to that based on total seed yield per plant. This indicates that the scores and rankings based on these two yield components can represent the evaluation of genotypic differences for salt tolerance in terms of total seed yield. The high correlation between these two yield components and total seed yield under salinity also indicates that spikelet and tiller numbers can predict seed yield better than other parameters.

A favourable combination of salt tolerance at seedling and seed maturity stages was identified in IR63731-1-1-4-3-2. This genotype possesses good yield potential due to high tillering ability and panicle weight, but has a relatively high stature and long growing period (G.B. Gregorio, personal communications, International Rice Research Institute, the Philippines). Our research suggests that this genotype should be introduced in a cross breeding program as an elite salt tolerant germplasm to incorporate different desirable agronomic traits. The other genotypes were either less tolerant or lacked a favorable combination of tolerance at different growth stages. For example, GZ5291-7-1-2 ranked as one of the most tolerant in terms of seedling growth, but as one of the least tolerant in terms of seed yield parameters. GZ178 ranked opposite to GZ5291-7-1-2 at early seedling and seed maturity stages. These results indicate that the salt tolerance mechanisms in these genotypes may be different at different growth stages.

A cluster analysis was used in this study to facilitate the evaluation of salt tolerance among genotypes. The major advantages of the utilization of a multivariate analysis in the evaluation for salt tolerance are the allowance of a simultaneous analysis on multiple parameters and the increase of the accuracy in the rankings of genotypes. Another advantage is the convenience to rank genotypes when plants are evaluated at different salt levels, e.g., moderate and high salt levels. Salt tolerance was overestimated, especially at high salt levels, when salt tolerance indexes were averaged across salt levels. This was pointed out in the study by Khrais et al. (1998). It was clearly shown in the present study that by simply adding the numbers in cluster group rankings at different salt levels, salt tolerance among genotypes can be estimated more conveniently and accurately. These advantages will be more obvious when a large number of genotypes have to be evaluated in salt tolerance breeding.

In conclusion, wide genotypic differences were observed for relative salt tolerance in terms of spikelet number per panicle and tiller number per plant. Spikelet and tiller numbers contributed most of the variations to seed yield under salinity among parameters investigated when data were averaged across all genotypes. Genotypic differences were also identified in the delay of reproductive growth, but this character was not strictly correlated with relative salt tolerance based on seed yield among genotypes. Dramatic changes of salt tolerance at early and seed maturity stages were observed in two genotypes, GZ5291-7-1-2 and GZ178. Only one genotype, IR63731-1-1-4-3-2, was identified with a favorable combination of salt tolerance at early and seed maturity stages. The method demonstrated in this study, i.e., cluster group ranking of genotypes based on multiple agronomic characters, can be applied in salt tolerance breeding to evaluate salt tolerance among genotypes with great advantage over conventional methods.

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